Electrophysiological Roles of L-Type Channels in Different Classes of Guinea Pig Sympathetic Neuron

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Davies, Philip J., David R. Ireland, Juan Martinez-Pinna and Elspeth M. McLachlan. Electrophysiological roles of L-type channels in different classes of guinea pig sympathetic neuron. *J. Neurophysiol.* 82: 818–828, 1999. The electrophysiological consequences of blocking Ca^{2+} entry through L-type Ca^{2+} channels have been examined in phasic (*Ph*), tonic (*T*), and long-afterhyperpolarizing (*LAH*) neurons of intact guinea pig sympathetic ganglia isolated in vitro. Block of Ca^{2+} entry with Co^{2+} or Cd^{2+} depolarized *T* and *LAH* neurons, reduced action potential (AP) amplitude in *Ph* and *LAH* neurons, and increased AP half-width in *Ph* neurons. The afterhyperpolarization (AHP) and underlying Ca^{2+}-dependent K^+ conductances (*g*KCa1 and *g*KCa2) were reduced markedly in all classes. Addition of 10 μM nifedipine increased input resistance in *LAH* neurons, raised AP threshold in *Ph* and *LAH* neurons, and caused a small increase in AP half-width in *Ph* neurons. AHP amplitude and the amplitude and decay time constant of *g*KCa1 were reduced by nifedipine in all classes; the slower conductance, *g*KCa2, which underlies the prolonged AHP in *LAH* neurons, was reduced by 40%. Surprisingly, AHP half-width was lengthened by nifedipine in a proportion of neurons in all classes; despite this, neuron excitability was increased during a maintained depolarization. Nifedipine's effects on AHP half-width were not mimicked by 2 mM Cs^+ or 2 mM anthracene-9-carboxylic acid, a blocker of Cl^- channels, and it did not modify transient outward currents of the A or D types. The effects of 100 μM Ni^{2+} differed from those of nifedipine. Thus in *Ph* neurons, Ca^{2+} entry through L-type channels during a single action potential contributes to activation of K^+ conductances involved in both the AP and AHP, whereas in *T* and *LAH* neurons, it acts only on *g*KCa1 and *g*KCa2. These results differ from the results in rat superior cervical ganglion neurons, in which L-type channels are selectively coupled to BK channels, and in hippocampal neurons, in which L-type channels are selectively coupled to SK channels. We conclude that the sources of Ca^{2+} for activating the various Ca^{2+}-activated K^+ conductances are distinct in different types of neuron.

INTRODUCTION

The somatic membranes of mammalian sympathetic postganglionic neurons bear several types of voltage-dependent Ca^{2+} channel that potentially are activated during the action potential (AP). In freshly dissociated somata of rat superior cervical ganglion (SCG), 80% of the current during action potential waveforms passes through N-type, 5% through L-type, and 1% through P-type channels; 14% of the current is resistant to blockade by known antagonists (Toth and Miller 1995). Q-type channels sensitive to ω-conotoxin MVIIC (McDonough et al. 1996) and low-voltage-activated T-type channels (Schofield and Ikeda 1988) have not been detected in rat SCG neurons. However T-type channels recently have been demonstrated in noradrenergic neurons of the rat pelvic ganglion (Zhu et al. 1995), indicating that sympathetic neurons in different locations can express distinct channel types. The populations of Ca^{2+} channels expressed in sympathetic neurons of other species have not been identified. Neither is it clear, even in central neurons, whether the Ca^{2+} channels expressed in somal membranes are representative of the entire population present in intact neurons as channel distribution can differ between soma and dendrites (Ahlijanian et al. 1990).

Ca^{2+} influx during the AP has electrophysiological consequences, particularly in sympathetic neurons, by modifying AP configuration (Belluzzi and Sacchi 1991; Davies et al. 1996) and initiating a prolonged afterhyperpolarization (AHP) (Cassell and McLachlan 1987b). All rat SCG neurons have a similar electrophysiological phenotype, firing phasically at the beginning of a maintained depolarization (Davies et al. 1996; Wang and McKinnon 1995). However, sympathetic neurons in the guinea pig behave in three distinctive ways when depolarized: phasic (*Ph*), tonic (*T*), and long afterhyperpolarizing (*LAH*) neurons have been distinguished (Keast et al. 1993; McLachlan and Meckler 1989). Major features of these classes of neuron are two distinct Ca^{2+}-activated K^+ conductances that underlie the AHP. Sympathetic neurons in all classes have a conductance, *g*KCa1, which is activated rapidly and decays with a time constant of 100–150 ms. *LAH* neurons have, in addition, a second, slower Ca^{2+}-activated K^+ conductance, *g*KCa2, that prolongs the AHP for several seconds (Cassell and McLachlan 1987b). This is largely mediated by Ca^{2+}-activated release of Ca^{2+} from internal stores (CICR) (Jobling et al. 1993).

Voltage-dependent Ca^{2+} influx also modifies the action potential itself. In rat SCG neurons (Davies et al. 1996), Ca^{2+} entering through L-type Ca^{2+} channels activates large conductance (BK type) Ca^{2+}-dependent K^+ channels contributing to AP repolarization so that blockade of L-type channels with nifedipine prolongs AP half-width. In contrast, the AHP follows activation of small conductance (SK type) Ca^{2+}-dependent K^+ channels primarily by Ca^{2+} influx through N-type channels. Ca^{2+} entering through Ca^{2+} channels resistant to all toxins prolongs the AHP in a proportion of rat SCG neurons by releasing Ca^{2+} from intracellular stores. Thus in these neurons, Ca^{2+} entry through particular types of channel has selective actions. However, we recently have found that N-type channels are involved in activation of both BK and SK channels in *Ph* neurons in the guinea pig lumbar paravertebral chain (Ireland et al. 1998).

In the present study, we have compared the physiological

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functions of Ca^{2+} entry in modifying the characteristics of the AP and AHP in the three classes of guinea pig sympathetic ganglion cell. We have focused on the effects of selective blockade of L-type channels using nifedipine. The data indicate that the electrical consequences of Ca^{2+} entry through L-type channels are distinctive for each class of neuron. They confirm that L-type channels in sympathetic neurons can admit quantities of Ca^{2+} sufficient to modify the AP itself, as well as the ensuing AHP, and that the coupling between Ca^{2+} channels and Ca^{2+}-activated K^+ channels in many guinea pig neurons differs from that in rat SCG neurons. Furthermore sympathetic neurons differ from hippocampal somata, in which L-type channels are coupled selectively only to SK channels (Marrion and Tavalin 1998).

METHODS

Guinea pigs (150–300 g of either sex) were deeply anaesthetized with pentobarbitone (80 mg/kg ip) and exsanguinated by perfusion through the descending thoracic aorta with oxygenated physiological salt solution. The SCG, lumbar paravertebral chain ganglia (LSC) between L_1 and L_6, coeliac ganglion or the inferior mesenteric ganglion and attached nerve branches were dissected and studied in different experiments. Ganglia were pinned out in vitro and superfused with oxygenated physiological salt solution at 35°C [composition (in mM): 151 Na^+, 4.7 K^+, 2.0 Ca^{2+}, 1.2 Mg^{2+}, 144.5 Cl^-, 1.3 $H_2PO_4^-$, 16.3 HCO_3^-, and 7.8 glucose, pH 7.2–7.4]. These procedures were approved by the Animal Care and Ethics Committee of the University of New South Wales. Intracellular recordings were made using microelectrodes filled with 0.5 M KCl (resistance 70–150 MΩ) and records taken in bridge mode, single-electrode current clamp (s.e.c.c), and single-electrode voltage clamp (s.e.v.c) as described in detail previously (Cassell et al. 1986; Davies et al. 1996).

Cells were classified as *Ph*, *T*, or *LAH*, and their passive electrical properties were determined as described previously (Cassell et al. 1986; Keast et al. 1993). Steady-state current-voltage relations from a holding potential of −60 mV were determined between AP threshold and values more negative than −90 mV near the end of a 250-ms hyperpolarizing current step. Passive electrical properties were determined at a holding potential of −60 to −65 mV where the current-voltage relationship was linear.

APs were generated after a brief (10–20 ms) depolarizing current step from resting membrane potential (RMP); they were differentiated digitally and threshold voltage for initiation of the action potential determined from the point at which the voltage differential (d*V*/d*t*) rose above 10 V/s. Because the RMP differed among classes, differences in the voltage threshold and amplitude of the AP and the amplitude of the AHP were compared using the absolute level of membrane potential. Depolarizations produced by drugs were compensated for by passing current to hold the potential at values more negative than −50 mV for measurements of APs. Effects on the repolarization phase were evaluated by integrating the area of the negative component of the differential (d*V*/d*t*), in some instances over only its latter part.

The time course of the AHP was defined by measuring its half-width (i.e., duration at half-peak amplitude). This measurement is very much dependent on the peak amplitude of the AHP: if the initial hyperpolarization is relatively large, the half-width may be brief even though there is a significant prolonged component (e.g., in *LAH* neurons). Unfortunately we found that other measures, such as integration of the voltage change during the AHP, were no more satisfactory than half-width as descriptors of the changes in AHP configuration. The time when the peak of the AHP occurred was measured from the point when the membrane repolarized to resting membrane potential until the peak of the afterpotential.

Outward tail currents were generated after a voltage command step (20–50 ms) that elicited only one "action current" (Cassell and McLachlan 1987b). The time course of *g*KCa1 in *LAH* neurons was derived by subtracting a function describing the sum of two exponentials fitted to *g*KCa2, from the overall tail current (see Cassell and McLachlan 1987b). The amplitude of *g*KCa1 was determined by extrapolating the exponential fitted to its decay except in a few cases in which the peak current fell below this value, when it was measured directly. The amplitude of *g*KCa2 was measured directly from the peak current. The maximum amplitude of the A-current (I_A) was measured at the end of a 250-ms voltage command step from −100 mV to a holding potential of −40 mV. Activation and inactivation of I_A and I_D were examined using standard protocols described previously (Cassell et al. 1986; Inokuchi et al. 1997).

RMP was measured as the difference between the potentials immediately before and after withdrawal of the microelectrode.

Drugs used

Nifedipine and anthracene-9-carboxylic acid (9AC) were obtained from Sigma (Castle Hill, NSW, Australia). Both were dissolved initially in ethanol and then diluted in physiological solution to reach the final working concentration. Care was taken when using nifedipine to minimize its exposure to light. Solutions containing drugs were added to the bath by transferring the inlet of the perfusion system to a solution containing the stated concentration. Effects of all drugs were recorded ≥15 min after this when a steady state of block had been achieved (see Davies et al. 1996). The effects of 9AC did not change after only 5-min exposure, although records were taken 10–20 min later. We have assumed in this study that addition of 10 μM nifedipine provides block of L-type Ca^{2+} channels. Unspecific blocking actions by nifedipine on Na^+ or K^+ channels are unlikely as there were no detectable changes in the amplitude of the AP overshoot that might reflect blockade of voltage-dependent Na^+ channels and in T and LAH neurons nifedipine had no effect on AP repolarization, suggesting it did not directly block BK, delayed rectifier or A-type channels involved in AP repolarization (Inokuchi et al. 1997).

Statistical analysis

All values are expressed as means ± SE. All recorded parameters were tested for equality of variance between groups (*F*-ratio and Bartlett's test, $\alpha = 0.05$) and then tested using appropriate parametric or nonparametric tests. Differences between properties in control and drug solutions were tested using a paired *t*-test or Wilcoxon signed-rank test. The effect of Ca^{2+} blockade or addition of nifedipine was compared between classes on the ratios of drug/control values using an ANOVA and multivariate ANOVA to determine any difference on the basis of all tests applied in the program, Superanova (Abacus Software, Berkeley, CA). Differences between groups were tested using an unpaired *t*-test or Mann-Whitney test. Differences in proportions of a particular trait among the three classes were tested using a χ^2 test, and the adjusted residuals were calculated to identify which classes were responsible for the significant overall χ^2 value. All reported significant differences had P values <0.05.

RESULTS

Electrophysiological properties of three classes of sympathetic neuron

RMP and passive electrical properties (Tables 1*A* and 2*A*) were similar to those previously reported (Cassell et al. 1986; Keast et al. 1993). The characteristics of the action potential and afterhyperpolarization are detailed in Tables 1, *B* and *C*, and 2, *B* and *C*.

TABLE 1. *Effects of blocking Ca^{2+} entry*

A. Passive electrical properties

	n	RMP, mV	R_{in}, MΩ	τ_{in}, ms	C_{in}, pF
Ph	15	−55 ± 1	145 ± 12	26 ± 3	192 ± 17
Ca^{2+} blockade		−52 ± 3	145 ± 22	23 ± 3	181 ± 17
P		0.16	0.69	0.55	0.21
T	10	−59 ± 3	149 ± 21	33 ± 4	244 ± 30
Ca^{2+} blockade		−53 ± 3*	146 ± 26	25 ± 2	193 ± 23
P		0.02	0.79	0.15	0.43
LAH	8	−59 ± 1	136 ± 22	17 ± 2	143 ± 21
Ca^{2+} blockade		−46 ± 3*	127 ± 15	15 ± 3	125 ± 20
P		<0.01	0.32	0.19	0.31

B. Action potential

	n	AP Threshold, mV	AP Overshoot, mV	AP Max d*V*/d*t*, V/s	AP Half-Width, ms
Ph	15	−27 ± 1	40 ± 3	211 ± 9	1.32 ± 0.05
Ca^{2+} blockade		−28 ± 1	36 ± 3*	189 ± 11*	1.53 ± 0.10*
P		0.73	0.02	<0.01	0.03
T	10	−30 ± 3	36 ± 3	217 ± 13	1.47 ± 0.13
Ca^{2+} blockade		−28 ± 2	36 ± 3	195 ± 10	1.45 ± 0.07
P		0.43	0.98	0.11	0.84
LAH	8	−30 ± 2	35 ± 2	191 ± 12	1.51 ± 0.13
Ca^{2+} blockade		−30 ± 2	27 ± 2*	159 ± 12*	1.45 ± 0.13
P		0.66	0.01	<0.01	0.72

C. Afterhyperpolarization

	n	Peak AHP, mV	AHP Half-Width, ms	*g*KCa1 Amplitude, pA	*g*KCal Decay τ, ms
Ph	15	−65 ± 1	110 ± 18	89 ± 14	109 ± 4
Ca^{2+} blockade		−57 ± 2*	51 ± 11*	34 ± 6*	55 ± 10*
P		<0.0001	<0.01	<0.001	<0.001
T	10	−68 ± 2	131 ± 17	113 ± 14	104 ± 12
Ca^{2+} blockade		−56 ± 1*	35 ± 5*	23 ± 6*	71 ± 12
P		<0.0002	<0.01	<0.001	0.13
LAH	8	−71 ± 1	83 ± 26	86 ± 9	119 ± 9
Ca^{2+} blockade		−60 ± 2*	18 ± 2*	24 ± 8*	66 ± 16*
P		0.001	0.04	<0.01	0.03

Effects of blockade of Ca^{2+} entry on passive membrane properties (*A*), the action potential (*B*), and the afterhyperpolarization (AHP) and underlying currents (*C*) in phasic (*Ph*), tonic (*T*), and long-afterhyperpolarizing (*LAH*) sympathetic neurons in control solution and after replacement of Ca^{2+} with 2 mM Co^{2+} or addition of 300 μM Cd^{2+}. Values are means ± SE. * $P < 0.05$. R_{in}, cell input resistance; τ_{in}, cell time constant; C_{in}, cell input capacitance.

Effect of blockade of Ca^{2+} entry

PASSIVE ELECTRICAL PROPERTIES. Ca^{2+} entry was blocked by replacement with Co^{2+} ($n = 25$), or addition of the nonspecific Ca^{2+} channel blocker, Cd^{2+} (300 μM; $n = 8$). There was significant membrane depolarization only in *T* and *LAH* neurons (Table 1*A*).

ACTION POTENTIAL. Overall, blockade of Ca^{2+} influx produced a significant decrease in the current required to reach threshold (control 0.38 ± 0.04 nA; Co^{2+}/Cd^{2+} 0.31 ± 0.03 nA; $n = 33$; $P = 0.01$) but the absolute threshold voltage at which the AP was initiated was unchanged (control −27 ± 2 mV; Co^{2+}/Cd^{2+} −28 ± 1 mV; $n = 33$; $P = 0.67$). However, in *Ph* and *LAH* neurons, both the overshoot of the AP and max d*V*/d*t* decreased (Fig. 1, Table 1*B*), presumably reflecting a reduction in total inward current.

AP half-width was increased only in *Ph* neurons. In control solution the repolarization phase of the AP often showed a shoulder which was reflected in a second negative-going phase in the d*V*/d*t* signal (Fig. 2, *Bb* and *Cb*). When such a shoulder was present (in about half of the neurons), it disappeared following Ca^{2+} channel blockade (Fig. 1, *B* and *C*). Correspondingly, the rate of repolarization (averaged over 2 ms after the onset of the second negative deflection, i.e., 0.74 ± 0.04 ms after the peak of the AP) was reduced (control 32.5 ± 1.0 V/s; Co^{2+}/Cd^{2+} 24.0 ± 1.0 V/s; $n = 33$; $P < 0.0001$).

The measured properties of the AP in T neurons was notably unaffected by blockade of Ca^{2+} entry (Table 1*B*).

AFTERHYPERPOLARIZATION. Blockade of Ca^{2+} entry markedly reduced the AHP amplitude (control 12 ± 1 mV; Co^{2+}/Cd^{2+} 6 ± 1 mV; $n = 33$; $P < 0.0001$) and its half-width (control 111 ± 12 ms; Co^{2+}/Cd^{2+} 39 ± 6 ms; $n = 33$; $P < 0.0001$). While the peak of AHP occurred earlier, this was not significant (time to peak AHP, control 17 ± 3 ms; zero Ca^{2+} 10 ± 2 ms; $n = 23$; $P = 0.06$). Under voltage clamp, peak amplitude of gKCa1 and its decay time constant were reduced. These changes were present in all classes although the reduction in

TABLE 2. *Effects of blocking L-type Ca^{2+} channels*

A. Passive electrical properties

	n	RMP, mV	R_{in}, MΩ	τ_{in}, ms	C_{in}, pF
Ph	20	−54 ± 1	198 ± 24	39 ± 3	220 ± 18
Nifedipine		−55 ± 1	222 ± 26	42 ± 3	215 ± 21
P		0.37	0.11	0.32	0.42
T	17	−60 ± 2	189 ± 30	38 ± 6	222 ± 21
Nifedipine		−60 ± 2	189 ± 27	37 ± 5	215 ± 19
P		0.59	0.94	0.57	0.40
LAH	12	−58 ± 1	151 ± 15	26 ± 2	179 ± 15
Nifedipine		−56 ± 1	170 ± 17*	28 ± 3	170 ± 15
P		0.06	0.03	0.10	0.41

B. Action potential

	n	AP Threshold, mV	AP Overshoot, mV	AP Max d*V*/d*t*, V/s	AP Half-Width, ms
Ph	20	−28 ± 1	40 ± 2	226 ± 8	1.16 ± 0.03
Nifedipine		−25 ± 1*	42 ± 3	218 ± 8	1.21 ± 0.03*
P		0.02	0.45	0.20	<0.001
T	17	−28 ± 1	40 ± 2	228 ± 7	1.41 ± 0.07
Nifedipine		−28 ± 1	39 ± 2	222 ± 9	1.42 ± 0.07
P		0.71	0.62	0.23	0.69
LAH	12	−29 ± 1	38 ± 2	214 ± 10	1.33 ± 0.04
Nifedipine		−25 ± 1*	39 ± 2	204 ± 9	1.33 ± 0.03
P		0.02	0.62	0.11	0.90

C. Afterhyperpolarization

	n	Peak AHP, mV	AHP Half-Width, ms	*g*KCa1 Amplitude, pA	*g*KCa1 Decay τ, ms
Ph	20	−66 ± 1	145 ± 15	115 ± 20	107 ± 4
Nifedipine		−64 ± 1*	164 ± 10	95 ± 16*	101 ± 6
P		0.04	0.11	0.02	0.13
T	18	−66 ± 4	107 ± 14	128 ± 17	97 ± 8
Nifedipine		−63 ± 4*	122 ± 12	111 ± 15*	86 ± 6*
P		0.004	0.07	0.01	0.04
LAH	12	−71 ± 1	111 ± 19	95 ± 7	111 ± 5
Nifedipine		−68 ± 1*	117 ± 19	81 ± 7*	96 ± 4*
P		0.02	0.63	0.04	<0.01

Effects of blockade of L-type Ca^{2+} channels on passive membrane properties (*A*), the action potential (*B*) and the AHP and underlying currents (*C*) in *Ph*, *T*, and *LAH* sympathetic neurons in control solution and after addition of 10 μM nifedipine to the perfusing solution. Abbreviations as in Table 1.

the decay time constant of gKCa1 was not significant in *T* neurons (Fig. 1, Table 1*C*). In *LAH* neurons, the long AHP and the slower component of the outward current, gKCa2, were virtually abolished by blockade of Ca^{2+} influx.

The effects of Ca^{2+} channel blockade were not different between the three classes of neuron (MANOVA, P = 0.60).

FIRING PROPERTIES. Following replacement of Ca^{2+} with Co^{2+}, the neurons became more excitable and fired a greater number of APs during a prolonged depolarizing current step. In *Ph* and *LAH* neurons, current steps that elicited a single AP in control solution fired multiple APs following Ca^{2+} blockade, whereas in T neurons, a given level of suprathreshold depolarizing current elicited repetitive firing at a higher frequency (see Fig. 8 in Davies et al. 1996).

Effect of blockade of L-type Ca^{2+} channels with 10 μM nifedipine

PASSIVE ELECTRICAL PROPERTIES. Addition of 10 μM nifedipine had no effect on passive membrane properties in *Ph* and *T* neurons whereas nifedipine caused a small increase in R_{in} in *LAH* neurons (Table 2*A*) which also depolarized slightly ($P = 0.06$). Current-voltage relations were not affected by nifedipine.

ACTION POTENTIAL. Nifedipine had no effect on the amplitude of the AP but threshold voltage was raised by 3–4 mV in *Ph* and *LAH* neurons (Table 2*B*). Max d*V*/d*t* was slightly reduced overall (control 224 ± 5 V/s; nifedipine 217 ± 5 V/s; $n = 49$; $P = 0.02$) (Fig. 1), although not significantly within any class (Table 2*B*). AP half-width was prolonged only in *Ph* neurons (by 4 ± 1%, Table 2*B*). The AP in *T* neurons was unaffected by nifedipine (Table 2*B*).

AFTERHYPERPOLARIZATION. Nifedipine reduced both AHP amplitude (control 12 ± 1; nifedipine 11 ± 1; $n = 50$; P = 0.0003) and the amplitude of gKCa1 (control 120 ± 10 pA; nifedipine 100 ± 10 pA; $n = 47$; $P = 0.0001$). In addition, in a subgroup of neurons with a relatively early AHP peak in control solution (<15 ms), nifedipine delayed the peak of the AHP (control 5 ± 1 ms; nifedipine 16 ± 3 ms; $n = 26$; $P = 0.0005$). In the remaining neurons with a slower onset AHP, nifedipine did not change the time at which the AHP peak

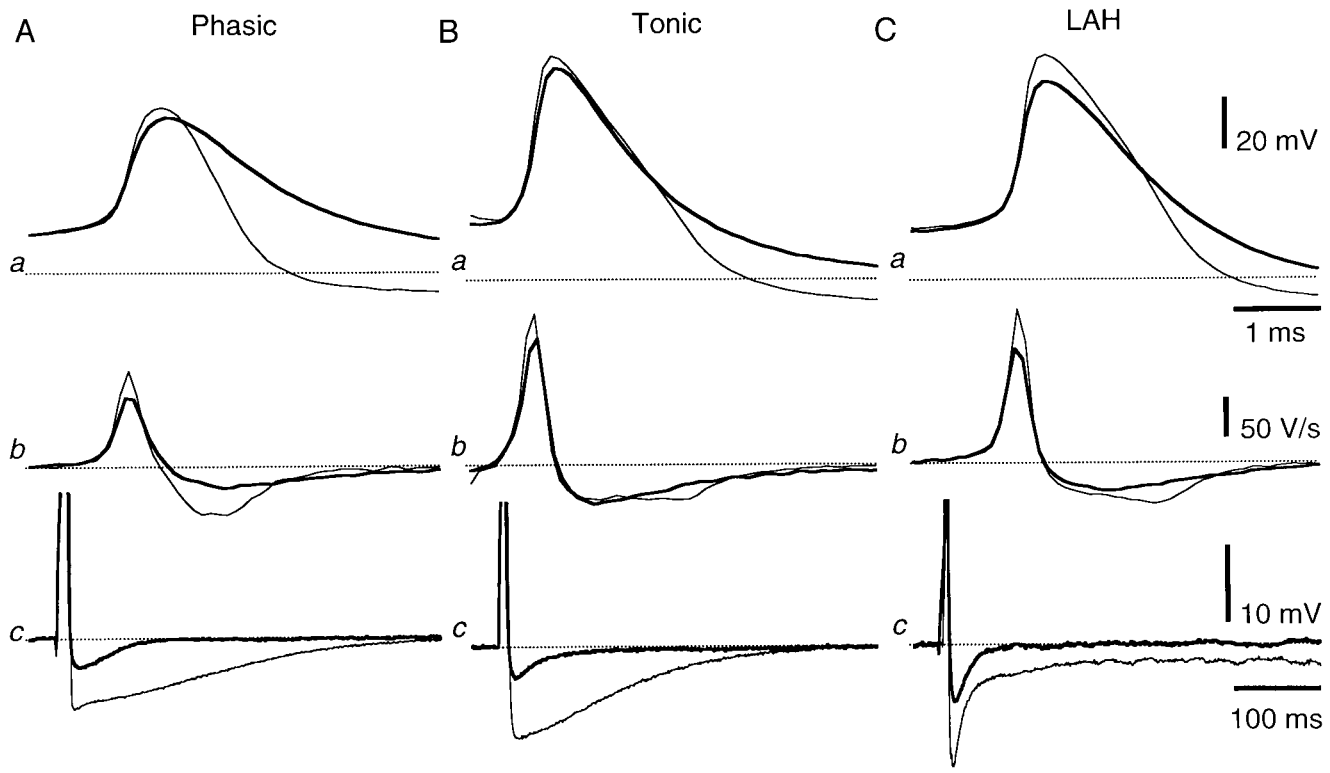

FIG. 1. Effects of blocking Ca^{2+} entry on the AP and AHP in *Ph* (*A*), *T* (*B*) and *LAH* (*C*) neurons. APs were evoked at resting membrane potential following a 10 ms depolarizing current step. Records show APs (*a*), their derivatives (d*V*/d*t*) (*b*) and AHPs following APs (truncated) on a slower time base (*c*). In this and subsequent figures, the traces recorded in control solution (thin line) and in test solution (thick line) have been overlaid. *A*: in a *Ph* neuron in the LSC, replacement of Ca^{2+} with 2 mM Co^{2+} caused a decrease in AP amplitude and marked slowing of its time course (*a*) so that both components of d*V*/d*t* were reduced in amplitude (*b*). *B*: in a *T* neuron in the CG, changing to Co^{2+} had little effect on the AP. However, the inflection on the repolarization phase was lost, and the later negative peak of the dV/dt disappeared (*b*). *C*: in an *LAH* neuron in the CG, Co^{2+} reduced AP amplitude and the predominant change in d*V*/d*t* was in the later negative component (*b*). Both peak amplitude and duration of the AHP were markedly reduced in neurons of all three classes (*c*); these records come from other neurons. Records were selected as showing changes close to the average for that class, including differences in RMP between classes. This also applies in subsequent figures. Scale bars in *C* apply throughout; the faster time scale applies to both *a* and *b*.

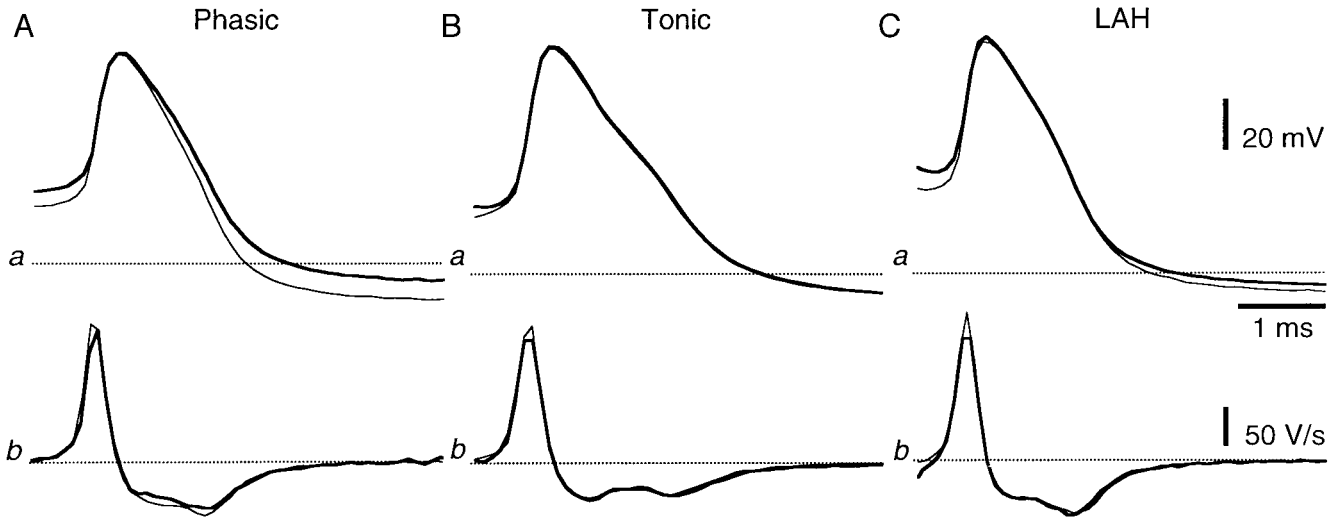

FIG. 2. The effects of 10 μM nifedipine on the AP in *Ph* (*A*), *T* (*B*) and *LAH* (*C*) neurons. Records show APs (*a*) and their derivatives (d*V*/d*t*) (*b*). *A*: in a *Ph* neuron in the SCG, nifedipine had no effect on AP amplitude but prolonged the repolarization phase (*a*). In addition, the threshold voltage at which the AP was initiated was raised and the negative component of d*V*/d*t* was slightly reduced (*b*). *B*: in a *T* neuron in the IMG, nifedipine did not modify the AP. *C*: in an *LAH* neuron in the CG, nifedipine raised the threshold voltage for AP initiation but had little effect on its shape. Scale bars in *C* apply throughout.

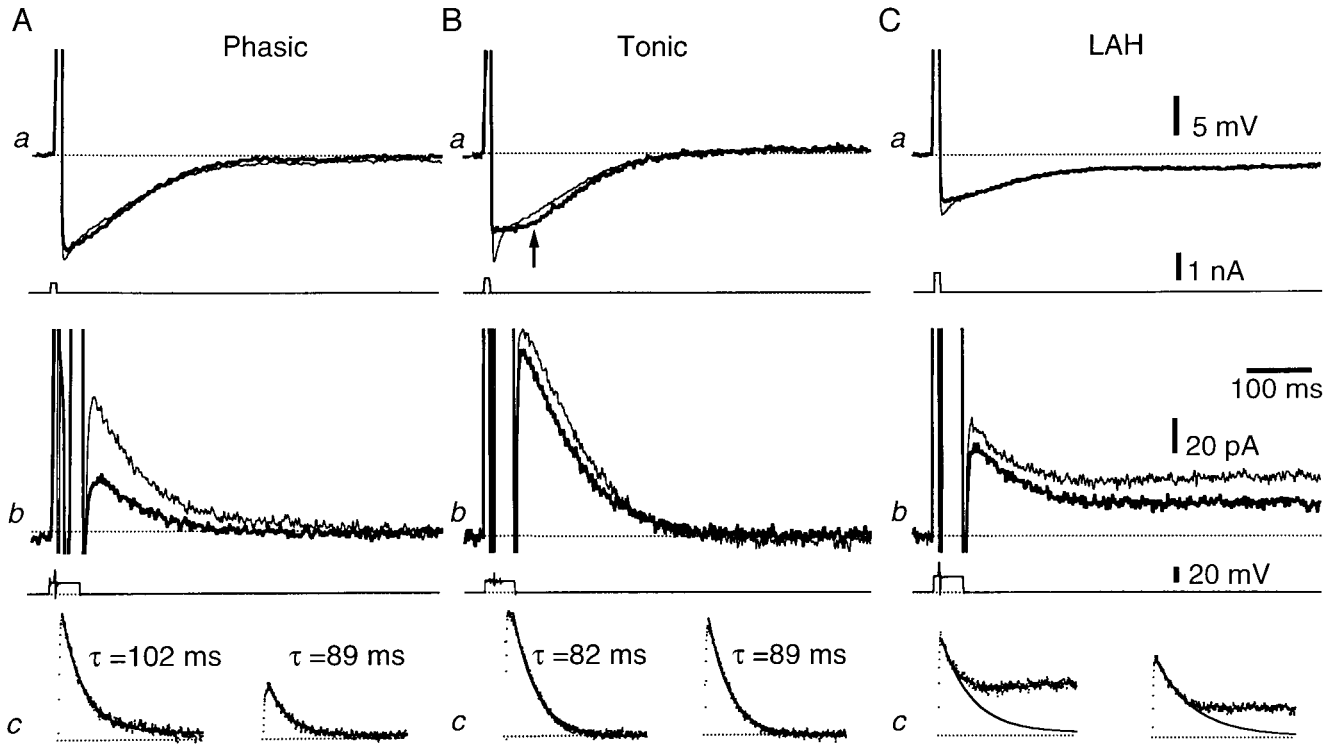

FIG. 3. Effect of 10 μM nifedipine on the AHP and gKCa1 in *Ph* (*A*), *T* (*B*) and *LAH* (*C*) neurons. (*a*) AHPs (*upper traces*) following APs (truncated) evoked by brief depolarizing current steps (*lower traces*) and (*b*) outward tail currents (*upper traces*) after action currents generated during depolarizing voltage commands (*lower traces*). In (*c*), the time courses of decay of tail currents are fitted by single exponentials (solid lines) in control solution (*left*) and in nifedipine (*right*) with time constants shown above each plot. In all neurons, the initial peak amplitude of the AHP was slightly reduced. *A*: in a *Ph* neuron in the IMG, although the time course of the AHP was barely affected, both amplitude and time constant of the tail current were reduced. *B*: in a *T* neuron in the IMG, the AHP widened by the appearance of a "belly" (arrow) although the tail current was slightly reduced. *C*: in an *LAH* neuron in the CG, nifedipine reduced only slightly the early part of the AHP and tail current but to a lesser extent than the prolonged component (see Fig. 5). Scale bars in *C* apply throughout.

occurred (control 45 ± 4 ms; nifedipine 43 ± 4 ms; $n = 23$; $P = 0.5$). The decay time constant of gKCa1 was briefer in nifedipine. These effects occurred to various extents in the different classes of neuron (Table 2*C*, Fig. 3).

Surprisingly, the overall effect of nifedipine was to lengthen the half-width of the AHP (control 123 ± 9 ms; nifedipine 137 ± 8 ms; $n = 50$; $P < 0.02$) (Fig. 3*Ba*). This widening occurred in a subpopulation of neurons in each class (*Ph* 13/20; *T* 12/18; *LAH* 6/12); in this subgroup, AHP half-width increased by 65 ± 21%. Widening of the AHP was generally accompanied by a reduction in the early fast component as well as the development of a wider "belly" on the slow component (Fig. 3) but there was either no effect or a reduction in gKCa1 amplitude and/or decay time constant. The passive electrical properties of this subgroup of neurons did not differ from those of the other neurons, nor was there any correlation between electrical properties and the effects of nifedipine on AHP half-width. Further, AHP amplitude was similar in these two groups and its decrease was similar in neurons with and without AHP widening, so that the change in half-width was not simply a consequence of the reduction in peak hyperpolarization.

The most prominent effect of nifedipine in *LAH* neurons was on the long AHP (Table 2*C*) and the prolonged conductance, gKCa2, underlying it. gKCa2 was reduced in amplitude by nearly 40% (control 33 ± 3 pA; nifedipine 20 ± 3 pA; $n = 10$; $P < 0.01$; Fig. 4) without modifying its time course (decay τ, control 1.28 ± 0.14 s; nifedipine 1.24 ± 0.28 s).

On the basis of its effects on RMP, R_{in}, AP half-width, AP threshold, AHP half-width and decay time constant of gKCa1, the effects of nifedipine were found to differ between the three classes of neuron ($P = 0.04$, MANOVA).

FIRING PROPERTIES. Addition of nifedipine had little effect on the firing properties during a just threshold depolarizing current step in any class of neuron, despite its actions in raising threshold. However in all neuron classes, the number of APs during larger current steps was usually greater than in control solution (Fig. 5). Thus the number of APs generated using a current step twice that necessary to trigger an AP was increased in all classes (*Ph* control 2.7 ± 0.6; nifedipine 4.2 ± 1.1; $n = 12$; $P = 0.04$; *T* control 3.1 ± 0.6; nifedipine 3.3 ± 0.5; $n = 14$; $P = 0.04$; *LAH* control 1.7 ± 0.3; nifedipine 2.8 ± 0.7; $n = 9$; $P = 0.02$).

Contribution of other Ca^{2+}-dependent channels to the AHP

The widening of the AHP by nifedipine, despite a reduction in gKCa1 (Table 2*C*), might be mediated by block of Ca^{2+} entry through channels that do not provide Ca^{2+} for activation of gKCa1. Two possible conductances that might normally attenuate the AHP were examined:

(I) EFFECTS OF BLOCKADE OF T-TYPE Ca^{2+} CHANNELS. T-type Ca^{2+} channels are blocked by 10 μM nifedipine (Randall and Tsien 1997). Activation of T-type Ca^{2+} channels during AHP repolar-

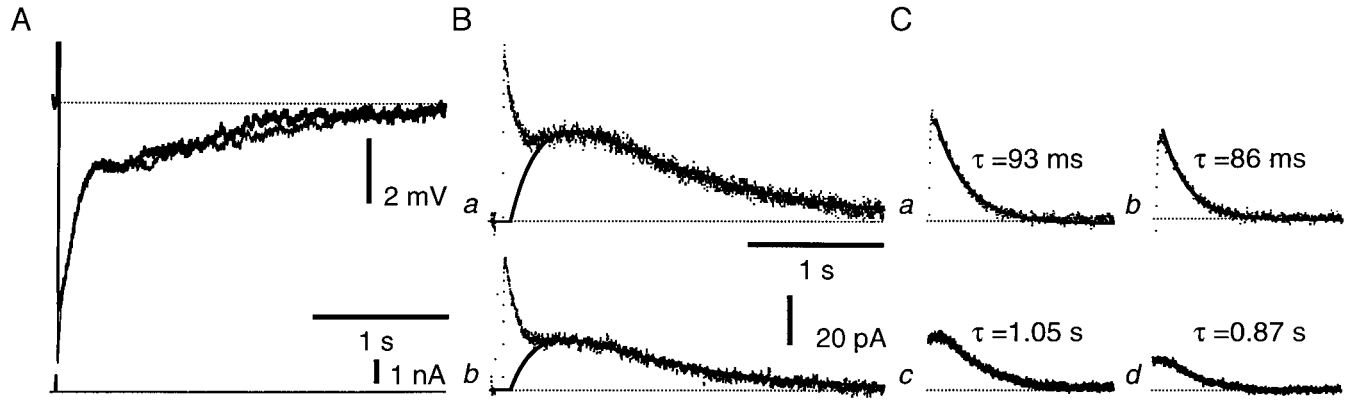

FIG. 4. Effect of 10 μM nifedipine on the AHP and slow outward tail currents in an *LAH* neuron. *A*: superimposition of AHPs in control and nifedipine solutions (*upper traces*) show that the later part of the AHP is reduced by nifedipine. The brief current steps used to generate the APs are shown in the lower trace. *B*: outward tail currents generated by single action currents in control solution (*a*) were reduced by nifedipine (*b*). The slower second components have each been fitted by a sum of two exponentials (solid lines) with τ_{on}= 265 ms and τ_{off} as shown in *c* and *d*. *C*: subtraction of the fits of the slow component reveals the initial tail currents in control solution (*a*) and in nifedipine (*b*) with single exponentials (solid lines) fitted to their decays (time constants shown above each plot). Similarly, the exponential fits to the decays of the slow component are shown in control solution (*c*) and in nifedipine (*d*). Nifedipine reduced the second slow component of the tail current (corresponding to gKCa2) more than the first component (gKCa1). This is the same *LAH* neuron as shown in Fig. 4*C*.

ization might attenuate its time course by introducing an inward current. We therefore tested the effects of 100 μM Ni^{2+}, a blocker of T-type Ca^{2+} channels (Fox et al. 1987). Addition of Ni^{2+} depolarized *T* neurons (control −62 ± 2 mV; Ni^{2+} −60 ± 1 mV, $n = 14$; $P = 0.01$) without significantly changing R_{in}. There was a small increase in AP half-width which was significant only in *T* neurons (*Ph*: control 1.34 ± 0.08 ms; Ni^{2+} 1.38 ± 0.09 ms, $n = 14$; *T*: control 1.52 ± 0.10 ms; Ni^{2+} 1.62 ± 0.09 ms, $n = 13$, $P = 0.01$; *LAH*: control 1.38 ± 0.14 ms; Ni^{2+} 1.40 ± 0.15 ms, $n = 6$), but there were no significant effects on the AHP or gKCa1 ($n = 33$). Addition of 10 μM nifedipine in the presence of 100 μM Ni^{2+} further increased AP half-width (control 1.23 ± 0.05 ms: Ni^{2+} 1.30 ± 0.05 ms; nifedipine 1.45 ± 0.09 ms, $n = 12$, $P = 0.02$). These data suggest that nifedipine's actions were not mediated by blockade of T-type channels.

(II) EFFECTS OF BLOCKADE OF Ca^{2+}-ACTIVATED CHLORIDE CHANNELS. In mouse SCG neurons, Ca^{2+} influx during the AP activates a Cl^- current producing an afterdepolarization (ADP) (De Castro et al. 1997) which attenuates the AHP. This ADP is blocked selectively by nifedipine, which does not affect gKCa1 after a single action potential (Martinez-Pinna et al. 1998). Application of the Cl^- channel blocker, 9AC (2 mM), had no effect on passive electrical properties or the AHP of 8 prevertebral *T* neurons (see also Clark et al. 1998). Neither did it affect the amplitude of gKCa1, although it decreased its decay time constant (control 118 ± 13 ms; 9AC 85 ± 9 ms; $n = 6$; $P = 0.03$). In one *T* neuron, in which 9AC had no effect on the AHP, addition of nifedipine subsequently widened it. These observations suggest that the actions of nifedipine on the AHP cannot be attributed to blockade of a Ca^{2+}-dependent Cl^- conductance.

Contribution of voltage-dependent K^+ currents to the AHP

The effect of nifedipine on AHP half-width is therefore most easily explained if it modulates a voltage-dependent outward

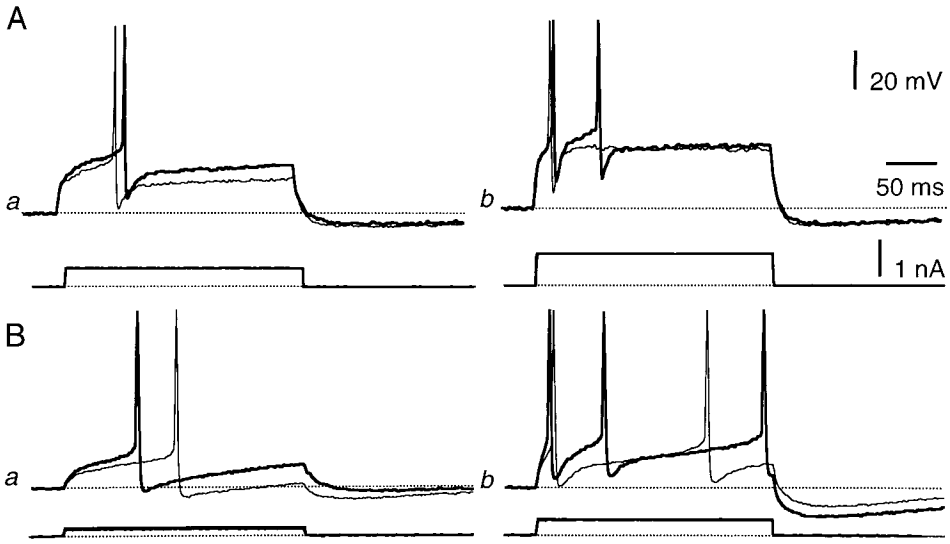

FIG. 5. Effect of 10 μM nifedipine on the responses to prolonged (250 ms) current steps in an *LAH* (*A*) and a *T* (*B*) neuron. Voltage response (*upper trace*) to a just-suprathreshold current step (*lower trace, a*) superimposed on the voltage response to a current step twice that amplitude (*b*), recorded in control solution (thin line) and in nifedipine (thick line). In (*b*), the current steps elicited more APs in nifedipine than in control solution. Scale bars in *A* also apply in *B*.

conductance activated during the AHP. We tested whether nifedipine had effects on either the A-current (I_A) which is particularly prominent in *T* neurons (Cassell et al. 1986), or the D-current, a slower transient outward current, which is also present in some *T* neurons (Inokuchi et al. 1997). However, I_A amplitude, time course and activation/inactivation characteristics were similar in control solution and in nifedipine ($n = 8$ *T* neurons). D-type currents which followed I_A in 7 *T* neurons were slightly reduced in amplitude (control 0.15 ± 0.03 nA, nifedipine 0.09 ± 0.01 nA; $P = 0.06$) and abbreviated (decay time constant: control 416 ± 54 ms; nifedipine 355 ± 56 ms; $P = 0.04$) in nifedipine, but activation and inactivation were unaffected. In addition, the presence of I_D was not correlated with widening of the AHP in nifedipine (χ^2 test, $P = 0.93$).

A time-dependent hyperpolarization-activated cationic conductance (I_H) attenuates the AHP in some central neurons (Schwindt et al. 1988). Addition of 2 mM Cs^+ had no effect on the shape or size of the AHP or gKCa1 in 3 *T* neurons. Further an effect on anomalous rectification is unlikely as the membrane potential during the peak of the AHP (see Tables 1 and 2) did not reach potentials at which significant rectification occurs. There was no correlation between the presence of inward rectification negative of −90 mV and the effect of nifedipine on the AHP (χ^2 test, $P = 0.38$).

Differences in the effects of nifedipine between ganglia

Ph neurons comprise the only class which is represented in different ganglia in sufficient numbers to test the possibility that the effects of nifedipine differ between anatomic locations. Among *Ph* neurons, nifedipine caused an increase in R_{in}(+31 ± 8%) in prevertebral neurons ($n = 5$, $P = 0.03$) that did not occur in paravertebral neurons (R_{in} +1 ± 7%, $n = 15$); this change was significantly different between the groups ($P = 0.01$). The change in AP half-width was similar in paravertebral (+4.2 ± 1.1%) and prevertebral (+4.9 ± 2.1%) neurons. In contrast, the amplitude of gKCa1 was significantly reduced by nifedipine in prevertebral (gKCa1 –34 ± 12%; $n = 5$; $P < 0.05$) but not in paravertebral neurons (gKCa1 −6 ± 5%, $n = 15$); these changes were significantly different ($P < 0.02$). Despite the larger reduction in gKCa1, widening of the AHP was generally greater in prevertebral *Ph* neurons (+191 ± 122%, $n = 5$; cf. *T* neurons, +25 ± 8%, $n = 18$) than in paravertebral ones (+10 ± 7%, $n = 15$, $P = 0.02$).

DISCUSSION

In this study, Ca^{2+} entry during the action potential has been shown to have distinct effects on the electrophysiological properties of each of the three main classes (*Ph, T, LAH*) of neuron present in guinea pig sympathetic ganglia. The new data on the consequences of Ca^{2+} entry provide confirmatory evidence for at least three main phenotypes of sympathetic neuron. In sympathetic neurons, voltage-dependent Ca^{2+} influx is responsible not only for the long AHP but, in *Ph* and *LAH* neurons, also determines the amplitude and time course of the AP itself. Both roles of Ca^{2+} are partly mediated by its influx through L-type Ca^{2+} channels, as revealed after selective blockade with nifedipine. In addition, nifedipine had two actions that were not seen when Ca^{2+} entry was blocked nonspecifically, namely, to raise the threshold for action potential initiation in *Ph* and *LAH* neurons and to widen the half-width of the AHP in some neurons. Overall, the results contrast with those of similar experiments on *Ph* neurons in the rat SCG in which Ca^{2+} influx through L-type channels led selectively to activation of BK-type channels during AP repolarization (Davies et al. 1996). However it should be noted that the effects of nifedipine in paravertebral *Ph* neurons were generally similar in both species.

The effects of Ca^{2+} entry through L-type channels on the configuration of the AP and the afterpotentials in sympathetic neurons (see also Martinez-Pinna et al. 1998) contrast with the lack of effect in postganglionic (Callister et al. 1997) and preganglionic parasympathetic neurons (Sah 1996) as well as in central neurons (e.g., Pineda et al. 1998) where influx of Ca^{2+} through L-type channels constitutes a significant proportion of the total Ca^{2+} current (Ishibashi et al. 1995). Following repetitive firing in hippocampal neurons, the AHP (Moyer et al. 1992; Norris et al. 1998) and the underlying slow Ca^{2+}-dependent K^+ conductance (Tanabe et al. 1998) are blocked by L-type channel antagonists although the APs themselves are unaltered. Recently, in patches of somatic membrane of hippocampal neurons, Ca^{2+} influx through L-type channels was shown to activate only SK -type channels despite BK-type channels being present within the same patch (Marrion and Tavalin 1998). This indicates that the sources of Ca^{2+} for activation of Ca^{2+}-sensitive channels are distinct in different neurons and that it is unlikely that diffusion is necessarily the determining factor.

Passive electrical properties

Blocking entry of extracellular Ca^{2+} depolarized the neurons by several mV (see also McLachlan 1977) but, in *T* neurons, this was not accompanied by a change in R_{in}, so that the effect probably did not involve a decrease in a resting Ca^{2+}-dependent K^+ conductance. However, depolarization of *LAH* neurons was accompanied by an increase in R_{in}. One explanation might be that gKCa2, which is activated by Ca^{2+} influx through L-type channels, is partly activated near RMP (see Tokimasa and Akasu 1995). The lack of effect of Cd^{2+}, however, indicates that other Ca^{2+}-sensitive mechanisms have opposing effects on resting conductance. As A-channels make an important contribution to RMP in *T* neurons (see Cassell et al. 1986; Inokuchi et al. 1997), it seems likely that resting membrane conductance is regulated by distinct mechanisms in each neuron class.

Action potentials

Several features of the AP were shown to be modified selectively by Ca^{2+} blockade:

1) Although threshold for AP initiation was not affected by Co^{2+} or Cd^{2+}, it rose by 2–3 mV in Ph and *LAH* neurons in nifedipine. A similar effect of nifedipine has been described in guinea pig hippocampal neurons (Higashi et al. 1990). This change is difficult to explain unless inward current through nifedipine-sensitive channels makes a significant contribution early in the regenerative response. Blockade of N-type channels with ω-conotoxin GVIA did not affect threshold in Ph neurons (Ireland et al. 1998).

2) The AP in *T* neurons was surprisingly unaffected by blockade of Ca^{2+} entry, compared with the effect on AP configuration in *Ph* and *LAH* neurons (see also Belluzzi and Sacchi 1991; Davies et al. 1996). In sympathetic neurons, at least three types of K^+ channels are activated during repolarization: delayed rectifier channels, A-channels (Belluzzi and Sacchi 1991; Inokuchi et al. 1997) and BK-type channels (Davies et al. 1996; Ireland et al. 1998). When Ca^{2+} entry was blocked, the inflection during the repolarization phase disappeared from all classes of neuron (as reported for other autonomic neurons, Callister et al. 1997; Mochida and Kobayashi 1986; Sah and McLachlan 1992; Yoshimura et al. 1986). This inflection is determined by the net addition of inward Ca^{2+} current and outward K^+ current so that block of Ca^{2+} entry has complex effects on repolarization. While the AP was widened by Co^{2+}/Cd^{2+} only in *Ph* neurons (by ~16%), only a small component (25%) of this change occurred via L-type channels. In *Ph* neurons, N-type channels play a much greater role in determining AP half-width but have no significant effects on amplitude and d*V*/d*t* (Ireland et al. 1998), implying that Ca^{2+} entry through channels other than N- or L-type must contribute. The failure of blockade of Ca^{2+} entry to affect AP half-width in *T* and *LAH* neurons suggests that these cells lack BK channels.

Slow afterhyperpolarizations

A major effect of blocking Ca^{2+} entry was to abolish the later phase of the AHP by inhibition of gKCa1 and gKCa2. The channels underlying gKCa1 are largely apamin-sensitive SK channels (Davies et al. 1996; Ireland et al. 1998; Jobling et al. 1993). In *Ph* neurons of the rat SCG (Davies et al. 1996), as well as in other autonomic (Callister et al. 1997; Sah 1996) and many central neurons (Pineda et al. 1992), most Ca^{2+} that activates SK channels enters via N-type Ca^{2+} channels. Of gKCa1, 60% depends on the Ca^{2+} provided via N-type channels in guinea pig paravertebral *Ph* neurons (Ireland et al. 1998); the present results indicate that ~15% of the rest comes through L-type channels (Tables 1 and 2) so that other Ca^{2+} channels must also contribute. In contrast, ~40% of gKCa2 depends on Ca^{2+} entry through L-type channels. This kinetically slow conductance is activated via CICR from intracellular stores (Jobling et al. 1993). The large effect of nifedipine on gKCa2 indicates that Ca^{2+} influx through L-type channels is important for activation of CICR (Li and Hatton 1997; Osmanovic and Shefner 1993).

Distribution of Ca^{2+} channels in sympathetic neurons

The effects of L-channel block on gKCa1 and gKCa2 are relatively large if it is assumed that the proportions of channels activated during the action potential in guinea pig neurons are the same as those in rat SCG somata, in which only 5% of the Ca^{2+} current is carried by L-type channels (Toth and Miller 1995). Perhaps the Ca^{2+} entering through L-type channels is much more effective in activating K^+ channels than the Ca^{2+} that enters through the more numerous N-type channels. This might result from the closer juxtaposition of L-type channels than N-type channels to the relevant K^+ channels. This explanation is hard to reconcile with the apparently rapid activation of gKCa1 by Ca^{2+} from both sources. Another explanation might be that the majority of L-type channels are located on dendrites and would not contribute to the current measured in dissociated somata. If this were the case, all types of K^+ channel that are activated by Ca^{2+} from L-type channels are probably also distributed over the dendrites. In this respect, in mouse SCG neurons, in which Ca^{2+}-activated Cl^- channels are thought to be located on distal dendrites (De Castro et al. 1997), nifedipine blocks their activation without affecting the SK channels underlying the AHP (Martinez-Pinna et al. 1998). In this context, it is interesting that L-type channel blockade had no effects on parasympathetic neurons which have few or very small dendrites (Callister et al. 1997).

Unexpected effects of nifedipine

Although nifedipine reduced both Ca^{2+}-activated K^+ conductances in most sympathetic neurons, it increased AHP half-width and generated a secondary "belly" in a subgroup, particularly prevertebral neurons. Such an effect did not occur when all Ca^{2+} influx was blocked. Nifedipine probably interfered with the regulation of a voltage-dependent conductance in all classes of neuron as the decrease in gKCa1 it produced was very rarely accompanied by a reduction in the AHP. We found no evidence for involvement of T-channels, Ca^{2+}-activated Cl^- channels or voltage-dependent K^+ channels of the A-, D- or H-types. Two other voltage-dependent K^+ currents might conceivably contribute to abbreviation of the AHP under normal conditions, namely, the time-independent inward rectifier, I_{IR} (Wang and McKinnon 1996) and the M-current (Selyanko and Brown 1996). Unfortunately we were unable to test these possibilities directly, as the appropriate antagonists, Ba^{2+} and muscarinic agonists, are not specific and also inhibit gKCa1 (Cassell and McLachlan 1987a). The consequences of L-type channel block in increasing excitability (Fig. 5) imply that the reduction in gKCa1 is more important functionally than AHP prolongation.

CONCLUSIONS

The results confirm that Ca^{2+} influx during the AP markedly affects the excitability of guinea pig sympathetic neurons of all classes, mainly by its effects on the slow Ca^{2+}-activated K^+ conductances (gKCa1 and gKCa2) that underlie the AHP. In this respect, the role of L-type channels differs from its roles in rat and mouse SCG neurons. Further, in *Ph* neurons of rats and guinea pigs, unlike other neurons so far studied, L-type Ca^{2+} channels are activated during a single action potential, providing Ca^{2+} that modifies repolarization through activation of BK channels. Thus Ca^{2+} entering through L-type channels activates BK, SK and possibly other K^+ channels in guinea pig sympathetic neurons (see also Wisgirda and Dryer 1994). The same diverse targets are modulated by the Ca^{2+} that enters through N-type channels (Ireland et al. 1998).

The present findings contrast with three examples of type-specific functional linkages: *1*) between L-type channels and BK channels, and between N-type channels and SK channels, in rat SCG cells (Davies et al. 1996; see also Wisgirda and Dryer 1994), *2*) between L-type channels and SK channels, and N-type channels and BK channels, in hippocampal neurons

(Marrion and Tavalin 1998) and *3*) between L-type channels and Cl^- channels, and again between N-type channels and SK channels, in mouse SCG cells (Martinez-Pinna et al. 1998). Our observations are not evidence against the concept that there is a close physical association between a voltage-dependent Ca^{2+} channel and the Ca^{2+}-sensitive channel(s) it activates. It might simply be that the pairing is not always specified by channel type. We conclude that the linkages are distinct in different types of neuron and hypothesize that associated pairs of channels may have specific distributions in different parts of the cell.

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